Canine Babesiosis

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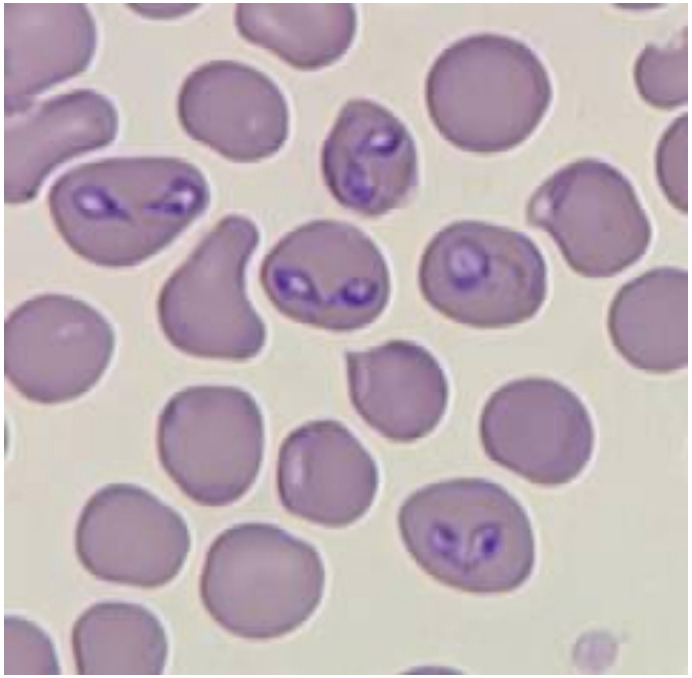
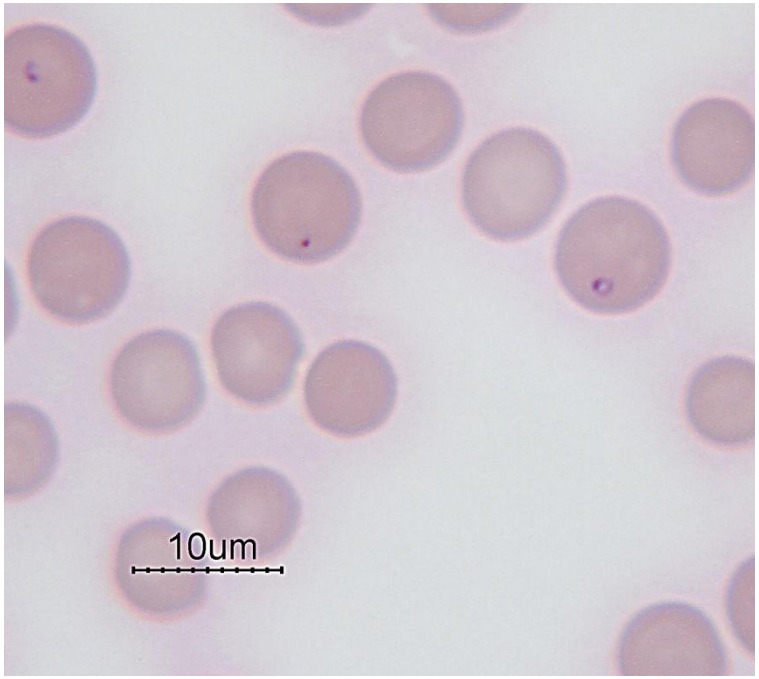
Babesiosis

- First tick-transmitted infection
- Smith and Kilbourne 1893
- Causative agent of “Texas Fever” in cattle
- How did they diagnose it?

Classification





Classification

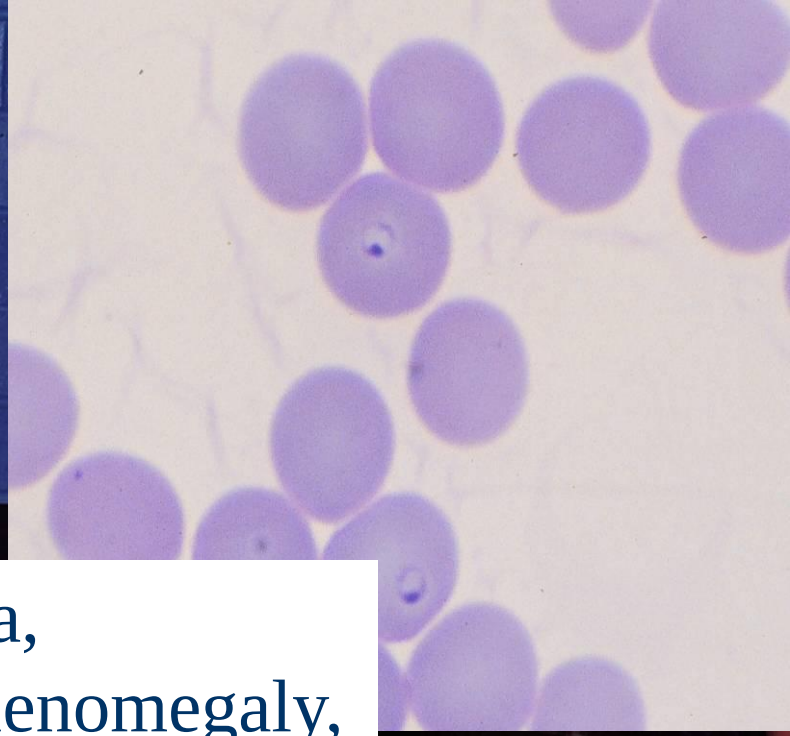
= *Babesia bovis*

= *Babesia canis*

History of canine babesiosis

- 1896: First case of canine babesiosis
- 1934: First canine case in USA
- 1968: First case of a small canine *Babesia* in USA
- 1983-1992: *Babesia canis* is prevalent in greyhounds
- 1991: First outbreak of canine babesiosis caused by small *Babesia*
- 100 years later, how were they diagnosed?

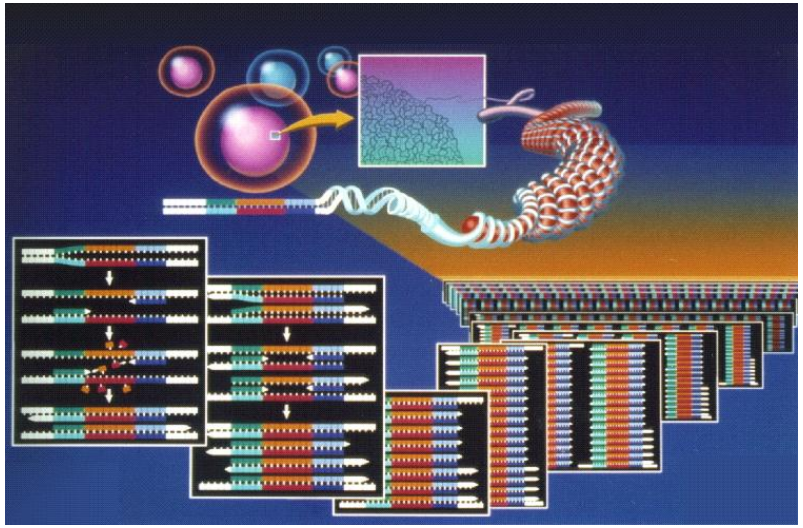
July 1, 1995:
My first day as a veterinarian



Hemolytic anemia, thrombocytopenia, hyperglobulinemia, icterus, fever, splenomegaly, and lymphadenopathy

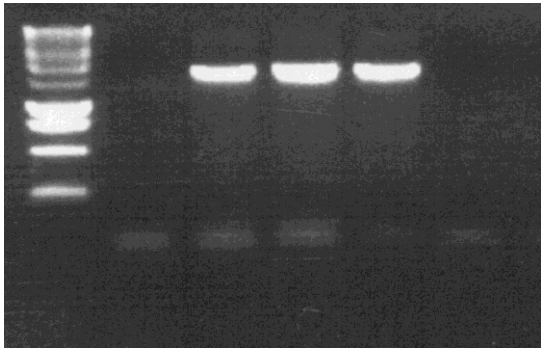
Polymerase Chain Reaction

- 1991: First *Babesia* DNA sequence in Genbank
- 1995: Two canine *Babesia* DNA sequences available
- *Babesia canis*
- *Babesia gibsoni*



What about our isolates?

- 3 Partial 18S rRNA gene sequences in GenBank for canine *Babesia* spp. in 1999
  - 2 *B. canis*, 1 *B. gibsoni* (from 1991 CA report)
- PCR primers designed by NCSU to differentiate *B. canis* and *B. gibsoni*
- Our "*B. gibsoni*" amplified with "*B. canis*" primers and NOT "*B. gibsoni*"



1 2 3 4 5 6

Lane 1: 1KB Molecular weight marker

Lane 2: canine DNA

Lane 3: *B. gibsoni* (Asian genotype)

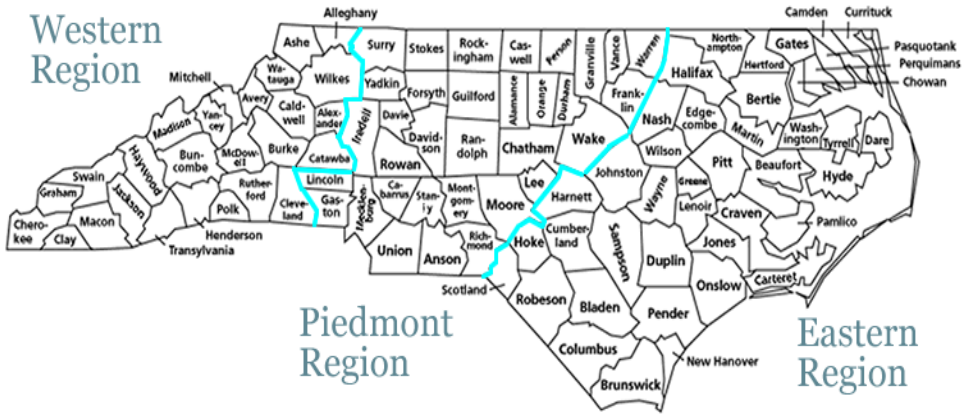
Lane 4: *B. c. canis*

Lane 5: *B. gibsoni* (California/USA genotype)

Lane 6: negative (no DNA) control

Epidemiology

Approach

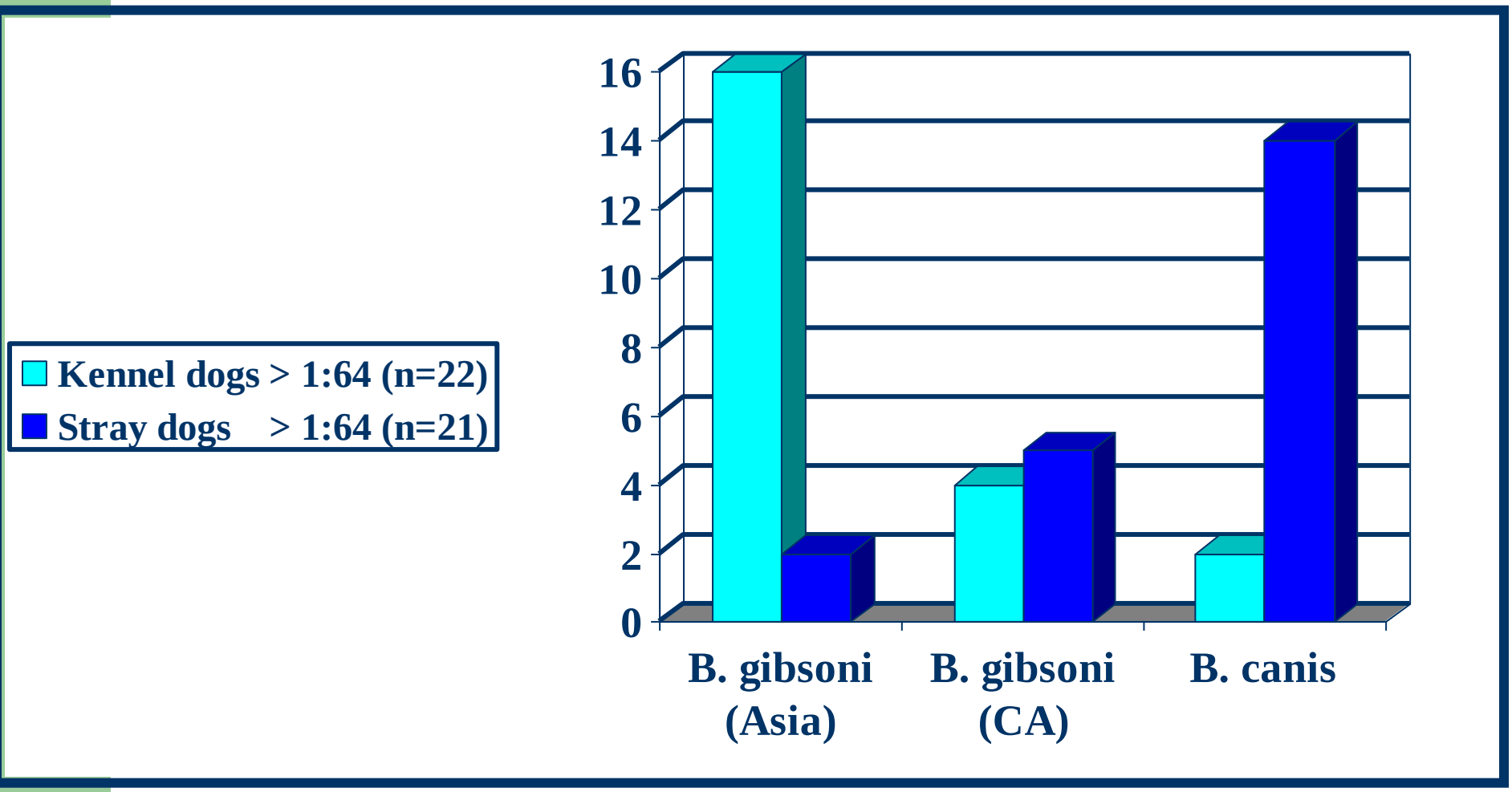


- Serologic and molecular survey:
  - Stray dogs in three geographic regions of NC
  - Three kennels where *B. gibsoni* infections had been diagnosed previously

Methods

- Samples:
- Stray: 359 dogs housed in animal shelters
  - Eastern NC: 168 dogs
  - Central NC: 140 dogs
  - Western NC: 51 dogs
- Kennel: 159 dogs housed in kennels where *B. gibsoni* infections had been diagnosed
  - Kennel I: 59 dogs
  - Kennel II: 43 dogs
  - Kennel III: 47 dogs

Results


Kennel dogs > 1:64 (n=22)
Stray dogs > 1:64 (n=21)
16
14
12
10
8
6
4
2
0
B. gibsoni (Asia)
B. gibsoni (CA)
B. canis

Results

- Microscopy Stray
  - 1 dog with small piroplasms
- Microscopy Kennel
  - 13 dogs small piroplasms
- PCR Stray
  - 1 *B. canis vogeli*
  - 2 *B. gibsoni* (Asian)
- PCR Kennel
  - 14 *B. gibsoni* (Asian)

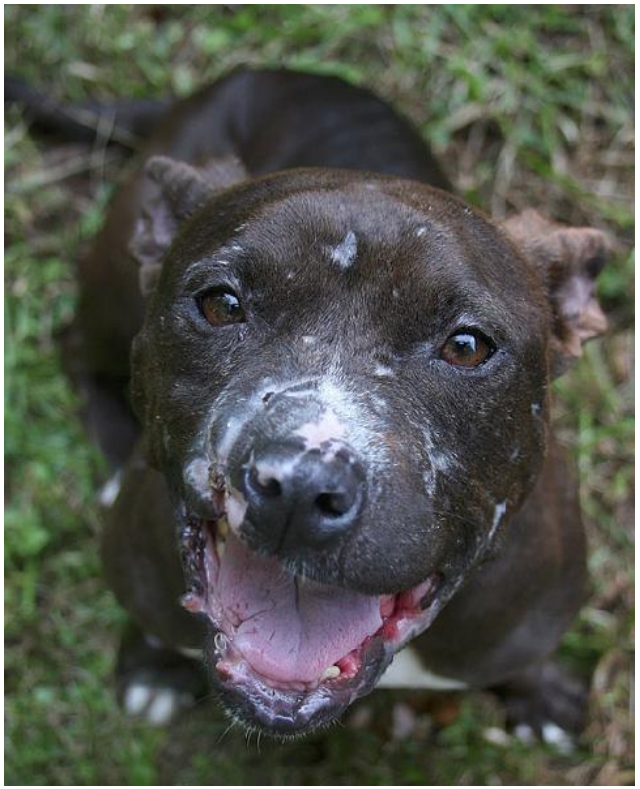
Conclusions

- *Babesia gibsoni* is endemic to NC
- Prevalence in kennels is high (6.8-25.6%)
- Seroreactivity may not accurately predict *Babesia* spp.
- Kennels comprised of American Pit Bull Terrier type dogs (96%)

(Birkenheuer et. al., JAAHA, 2003)

Hypothesis:

- Dogs that test positive for *B. gibsoni* are more likely to be American pit bull terriers



Approach

- Retrospective analysis samples submitted to the NCSU-Vector Borne Disease Diagnostic Laboratory for *Babesia* PCR between May 2000 and October 2003

Results

- 688 canine submissions were reviewed
  - Geographic location
  - Breed
- 145/688 (21%) samples tested positive for the presence of *Babesia* spp. DNA

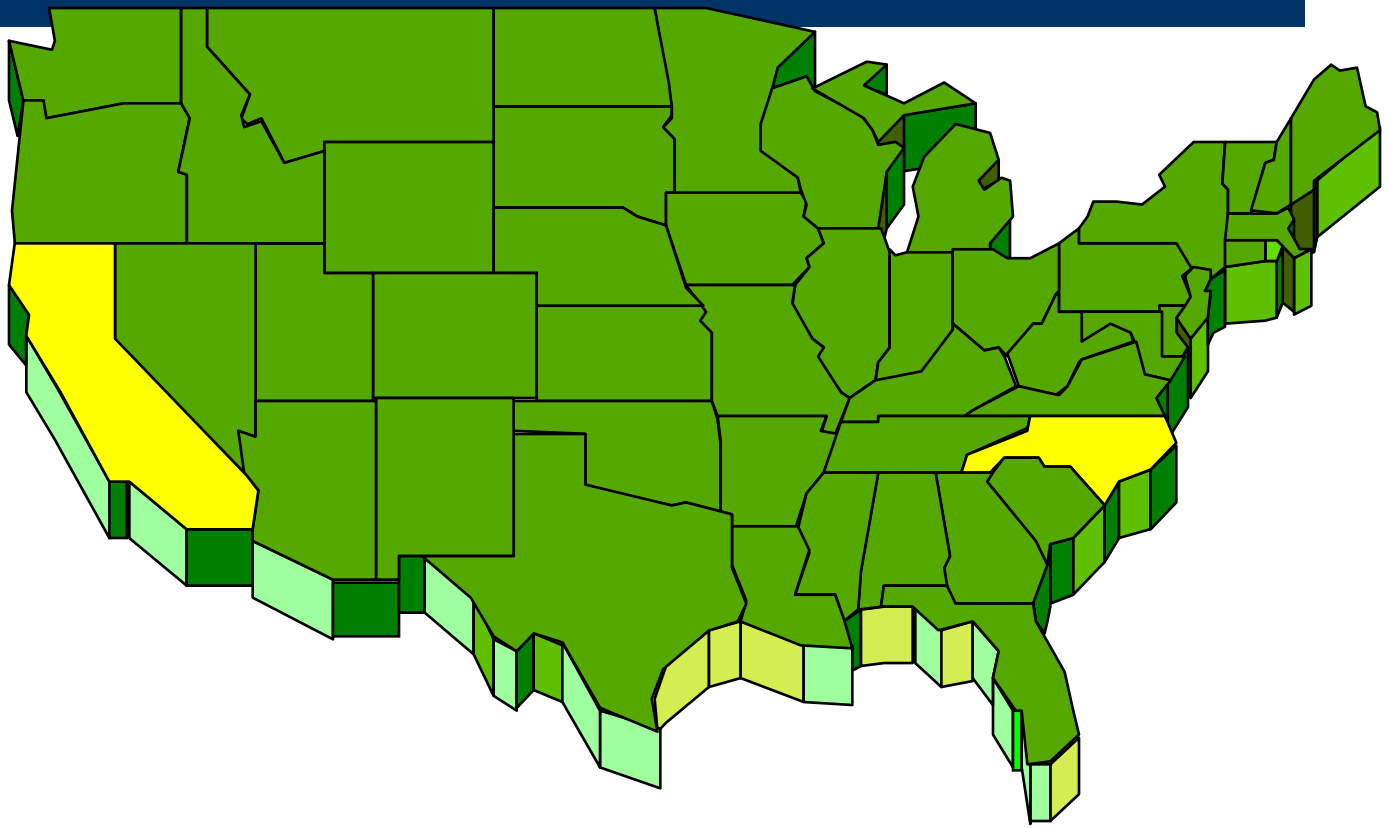
Results

	Babesia gibsoni	*Babesia canis vogeli*	Other piroplasm
Total	**131**	**11**	**3**
APBT*	**121**	**0**	**1**
Greyhound	**0**	**8**	**0**
Other Breed	**10**	**3**	**2**

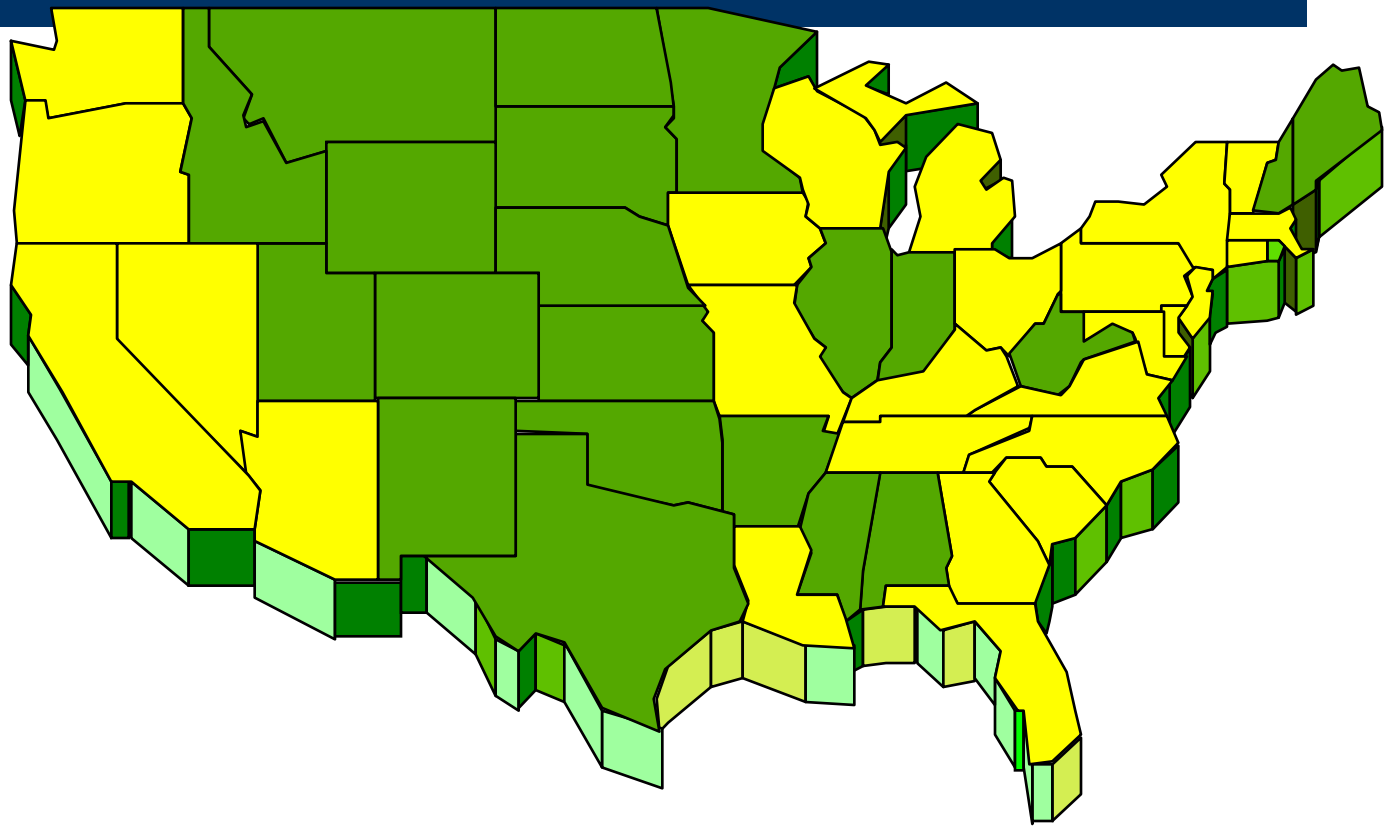
* APBT: American Pit Bull Terrier


freecodesource.com

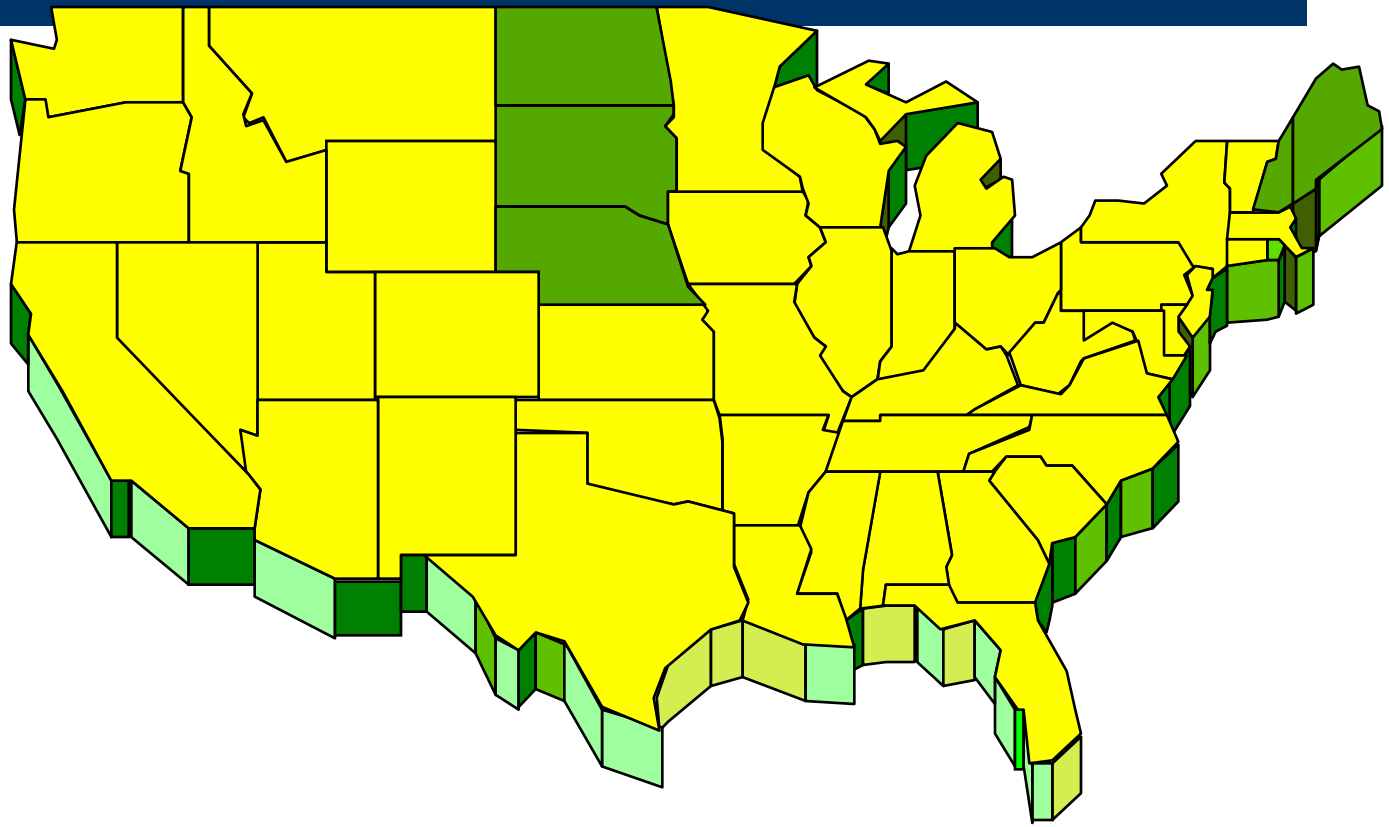
How extensive is the infection: 1995



How extensive is the infection: 2003



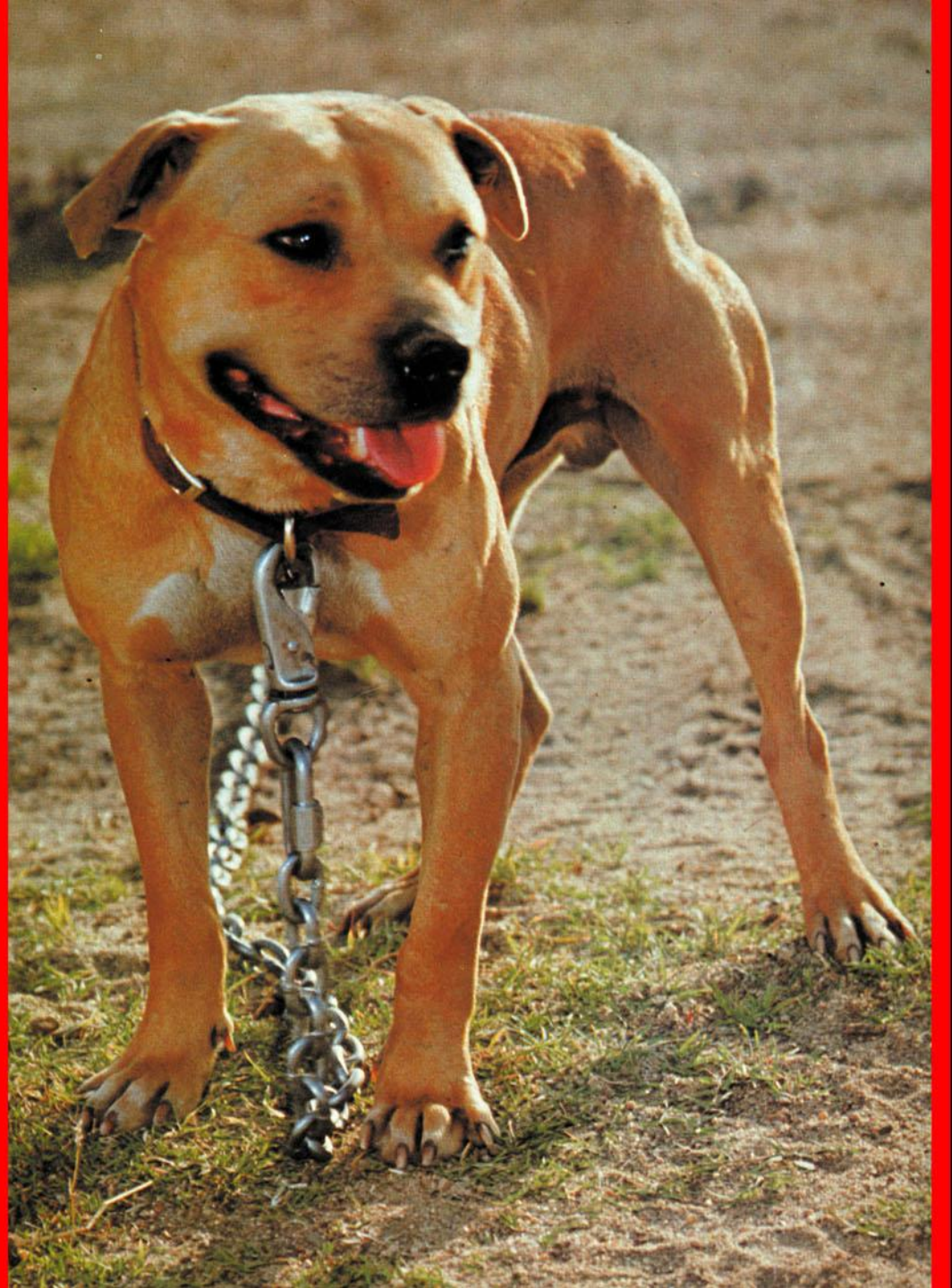
How extensive is the infection: 2010



Results

- Dogs testing positive for *Babesia* spp DNA were more likely to be APBT dogs compared to all other dogs tested (Odds ratio 12.7; 95% CI 7.66 to 21.20; $p < 0.0001$)
- Dogs testing positive for *B. c. vogeli* DNA were more likely to be Greyhounds compared to other non-APBT dogs tested (Odds ratio 4.96; 95% CI 1.08 to 25.51; $p = 0.02$)
- *Babesia gibsoni* is widespread in USA

Typical living conditions of the kennel dogs



Hypothesis

- *Babesia gibsoni* infections in the US are transmitted directly via dog bites and are not vector-transmitted
- APBT owners were not willing to give out information about previous bite wounds
- *Babesia gibsoni* infections in non-pit bull dogs are associated with a recent dog bite

Approach

- Retrospective analysis of medical records of non-APBT dogs confirmed to have *B. gibsoni* infection identified by PCR in the VBDDL at NCSU
- To determine whether or not exposure to ticks or a history of a fight with an American Pit Bull Terrier were risk factors for *B. gibsoni* infection

Results

- 15 *B. gibsoni* infected dogs that were not APBTs were identified
  - 3 excluded due to lack of known history
- 12 *Ehrlichia ewingii* infected dogs were used as a control group representing dogs with a tick-transmitted disease

	Dog bite	APBT* bite	Ticks	Housed with infected dog
Babesia gibsoni	**9/12**	**9/12**	**2/12**	**4/12**
Ehrlichia ewingii	**3/12**	**0/12**	**5/12**	**2/12**

* APBT: American Pit Bull Terrier

Results/Conclusions

- *B. gibsoni* infected dogs were more likely to have had a recent dog bite than *E. ewingii* infected dogs (Odds ratio 9.0; 95% CI 1.07 to 96.34; p = 0.014)
- The association was stronger ($p < 0.0001$) when the dog inflicting the bite was an APBT
- The association remained ($p = 0.045$) when each household with multiple infected dogs was counted as a single case

Now What?

- Developed test
- Identified epidemic
- Identified risk factors for infection
- THERE IS NO EFFECTIVE TREATMENT FOR *Babesia gibsoni*!

Treatment

- Treatments reduce morbidity and mortality but cannot clear the infections
  - Imidocarb, diminazene, trypan blue, phenamidine, doxycycline, clindamycin….
- Treated dogs are carriers and are at risk for recurrence of signs and can transmit infections to other dogs

Atovaquone and Azithromycin

- Atovaquone is a hydroxy-1,4-naphthoquinone: analog of ubiquinone and interferes with electron transport
- Azithromycin is an azalide antibiotic and interferes with protein synthesis
- Both drugs have anti-*Babesia* activity
- Together have been effective against other *Babesia* spp.

(Hughes 1995, Wittner 1996, Pudney 1997, Gray 1999, Krause 2000)

Specific Aim

- To determine whether or not an atovaquone and azithromycin drug combination would decrease *Babesia gibsoni* parasitemia below the limit of detection

Approach

- Double-blind placebo-controlled
  - 11 treatment
  - 11 placebo
- Could detect 60% difference between groups (based on pilot study)
- 3 post-treatment sample dates
- Detection Semi-nested PCR that can detect 50 organisms/ml
- CBC, biochemical profiles

Results

Dog	Group	Day 0	Day 60	Day 90	Day 120
1	Treatment	+	-	-	-
2	Treatment	+	-	-	-
3	Treatment	+	-	-	ND
4	Treatment	+	-	-	-
5	Treatment	+	-	-	-
6	Treatment	+	+	+	ND
7	Treatment	+	-	-	-
8	Treatment	+	-	-	-
9	Treatment	+	-	-	-
10	Treatment	+	-	-	-
11	Treatment	+	-	+	ND
12	Placebo	+	+	+	+
13	Placebo	+	+	ND	ND
14	Placebo	+	+	+	-
15	Placebo	+	+	+	+
16	Placebo	+	+	+	ND
17	Placebo	+	-	+	+
18	Placebo	+	+	+	+
19	Placebo	+	-	+	+
20	Placebo	+	+	+	+
21	Placebo	+	+	-	+
22	Placebo	+	+	+	+

+, positive PCR test
-, negative PCR test
ND, not determined

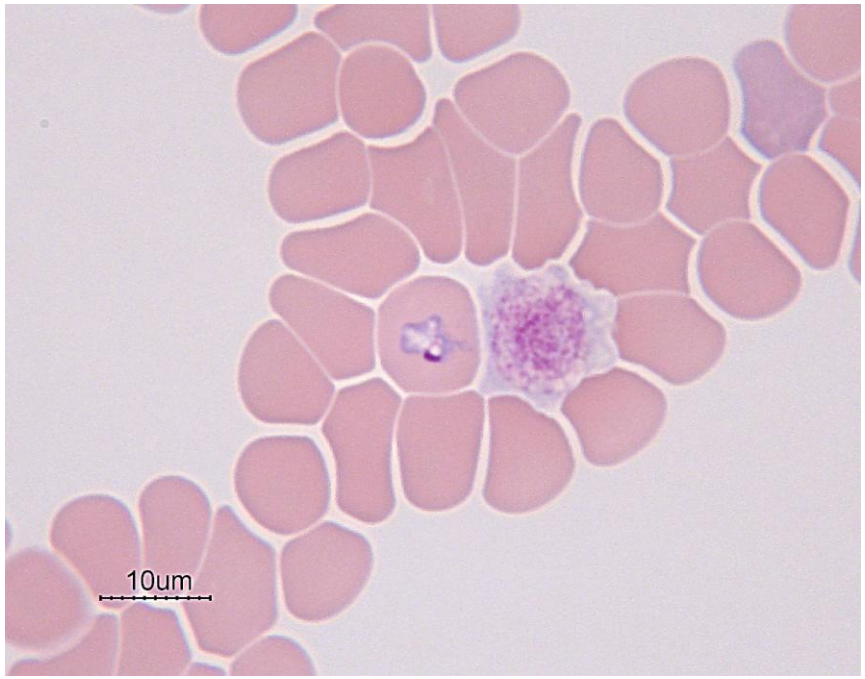
Results/Conclusions

- There was a significant difference between the treatment and placebo groups (p= 0.0001)
- No side-effects were reported in either the treatment or placebo treated dogs
- Only treatment reported to eliminate detectable *B. gibsoni* parasitemia

(Birkenheuer et. al., J Vet Internal Med, 2004)

There are currently at least 9 genetically unique canine piroplasms

1. *B. gibsoni*
2. *B. vogeli*
3. *Babesia sp. coco*
4. *B. conradae*
5. *B. canis*
6. *B. rossi*
7. *T. annae*
8. Novel sp. in England
9. *T. equi*
10. There will be more!



Conclusions

- There is no microscope objective powerful enough to decipher DNA sequence
- There are currently 745,314 *Babesia* DNA sequences in Genbank
- We are probably just seeing the tip of the iceberg
- *Babesia* are not as “species-specific” as we think
- Humans are a good model for animal disease

Questions?